Review

The thalamus of the monotremes: cyto- and myeloarchitecture and chemical neuroanatomy

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Echidna and platypus brains were sectioned and stained by Nissl or myelin stains or immunocytochemically for calcium-binding proteins, gamma aminobutyric acid (GABA) or other antigens. Cyto- and myeloarchitecture revealed thalami that are fundamentally mammalian in organization, with the three principal divisions of the thalamus (epithalamus, dorsal thalamus and ventral thalamus) identifiable as in marsupials and eutherian mammals. The dorsal thalamus exhibits more nuclear parcellation than hitherto described, but lack of an internal medullary lamina, caused by splaying out of afferent fibre tracts that contribute to it in other mammals, makes identification of anterior, medial and intralaminar nuclear groups difficult. Differentiation of the ventral nuclei is evident with the ventral posterior nucleus of the platypus enormously expanded into the interior of the cerebral hemisphere, where it adopts a relationship to the striatum not seen in other mammals. Other nuclei such as the lateral dorsal become identifiable by expression of patterns of calcium-binding proteins identical to those found in other mammals. GABA cells are present in the ventral and dorsal thalamic nuclei, and in the ventral thalamus form a remarkable continuum with GABA cells of the two segments of the globus pallidus and pars reticulata of the substantia nigra.

Keywords: platypus; echidna; dorsal thalamus; ventral thalamus; epithalamus; GABA cells

1. INTRODUCTION

The thalamus has a fundamentally similar pattern of nuclear organization and connectivity in all mammals. The basic divisions of the thalamus into ventral thalamus, dorsal thalamus and epithalamus can be readily identified in all metatherian and eutherian species, even in those in which the component nuclei of these divisions are least differentiated (Jones 1985, 2007). In two of the three living monotremes, the platypus and short-beaked echidna, the cytoarchitecture of the thalamus has been described as being rather monotonous, the nuclear divisions being less evident than in marsupials and eutherian mammals. The monotreme thalamus as a consequence has come to be regarded as less differentiated than that of other mammals (Ziehen 1897; Hines 1929; Abbie 1934; Lende 1964; Campbell & Hayhow 1971, 1972; Welker & Lende 1980; Ulinski 1984). The thalamus of the third living monotreme species, the rare long-beaked echidna, has not been examined. The ancestors of the monotremes are thought to have split off from the line ancestral to metatherian and eutherian mammals *ca* 150 Myr ago, with the fore-runners of marsupials and eutherian mammals only separating from a common stock much later at *ca* 100 Myr ago (e.g. Radinsky 1987; Musser 2003; Van Rheede *et al.* 2006). The supposedly undifferentiated

thalamus of the living monotremes, therefore, could potentially provide clues to what may have been the ancestral state of the thalamus at a time when, in the course of evolution, the earliest reptile-like mammals had emerged.

Recent experimental work devoted to mapping the principal sensory and motor areas of the cerebral cortex of the platypus and echidna shows that despite some significant differences in the degree to which these areas can be delineated from one another, they are laid out in a manner very comparable to that found in other mammals (Rowe 1990; Krubitzer *et al.* 1995; Manger *et al.* 1996). All evidence points to the parcellation of the mammalian cerebral cortex into functional areas occurring in close conjunction with a similar differentiation of the thalamic nuclei that provide input to the cortical areas (Jones 1985, 2007). Hence, there may be a far greater degree of nuclear differentiation in the monotreme thalamus than hitherto assumed. There have been no comprehensive studies of the structure of the thalamus of the extant monotremes that would permit the necessary re-evaluation, and none carried out with the full armamentarium of neuro- and chemoanatomical labels that have proven so valuable in extending our knowledge of the structural and functional organization of the thalamus of the common experimental animals. Recent partial study of the thalamus of the echidna indicates that it is possible to apply some of the more common histo- and immunocytochemical staining techniques to the monotreme thalamus (Ashwell & Paxinos 2005) and implies that a more

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Table 1. List of abbreviations.

A	anterior nuclei of thalamus
AC	anterior commissure
Am	amygdala
CALB	calbindin
Ce	central nuclei
CG	central gray
CP	cerebral peduncle
F	fornix
FF	field of Forel
GPe	external division of globus pallidus
GPI	internal division of globus pallidus
HC	habenular commissure
HL	lateral habenular nucleus
Hm	medial habenular nucleus
HPT	habenulopeduncular tract
Hy	hypothalamus
IC	internal capsule
IP	interpeduncular complex
IPC	interpallidal commissure
LD	lateral dorsal nucleus of thalamus
LGd	dorsal lateral geniculate nucleus
LGv	ventral lateral geniculate nucleus
LM	medial lemniscus
?LP	possible lateral posterior nucleus of thalamus
M	medial nucleus of thalamus
?MG	possible medial geniculate complex
MTT	mammillothalamic tract
MV	medioventral nucleus of thalamus
NST	nucleus of stria terminalis
OT	optic tract
Pa	paraventricular nucleus of epithalamus
PARV	parvalbumin
PC	posterior commissure
Pi	pineal body
Pf	parafascicular nucleus of thalamus
PT	pretectum
Pt	parataenial nucleus of thalamus
R	reticular nucleus of thalamus
S	striatum
Sb	subthalamic nucleus
SC	superior colliculus
SM	stria medullaris
SNc	pars compacta of substantia nigra
SNr	pars reticulata of substantia nigra
ST	stria terminalis
VL	ventral lateral nucleus of thalamus
VM	ventral medial nucleus of thalamus
VMb	basal ventral medial nucleus of thalamus
VP	ventral posterior nucleus of thalamus
VT	vena terminalis
VTa	ventral tegmental area
ZI	zona incerta
?	unknown dorsal thalamic nucleus
??	second unknown thalamic nucleus

complete analysis with a broader range of techniques would be profitable.

2. MATERIAL AND METHODS

This study was carried out on the brains of three platypuses (*Ornithorhynchus anatinus*) and four short-beaked echidnas (*Tachyglossus aculeatus*). The brains from two of the platypuses and two of the echidnas came from animals recently caught in the wild in Queensland, Australia, with the appropriate permits

(see Siegel *et al.* 1996, 1999 for details). The remaining brains came from an archive of already sectioned material in the collection of one of the authors (E.G.J.).

The newly caught animals were anaesthetized with 30 mg kg⁻¹ ketamine and 1.3 mg kg⁻¹ xylazine injected intramuscularly, given a lethal dose of intraperitoneal sodium pentobarbital, and perfused through the heart with normal saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed, infiltrated with phosphate-buffered sucrose and later frozen in dry ice. All procedures on the animals prior to sectioning the brains were carried out at the University of Queensland following the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Staining. The new brains were sectioned serially at a thickness of 30 µm on a sliding microtome and the sections collected in groups of five in 0.1 M phosphate buffer. One platypus brain and one echidna brain were sectioned in the frontal plane, and one platypus brain and one echidna brain were sectioned in the parasagittal plane. From each brain, a one-in-five series of sections was stained with thionin, a one-in-ten series was stained with haematoxylin, and one-in-five or one-in-ten series were stained for acetyl cholinesterase or cytochrome oxidase activity or immunocytochemically for parvalbumin, 28 kDa calbindin, 29 kDa calretinin, tyrosine hydroxylase, serotonin, gamma aminobutyric acid (GABA), α -type II calcium/calmodulin-dependent protein kinase or with the monoclonal antibody, SMI-32. The following primary antibodies or antisera were used: parvalbumin (Sigma, 1 : 1000); calbindin (provided by Dr P. C. Emson, 1 : 2000); calretinin (Chemicon, 1 : 2000); tyrosine hydroxylase (Eugene Tech, 1 : 500); serotonin (Sigma, 1 : 4000); GABA (Sigma, 1 : 10 000); α -type II calcium/calmodulin-dependent protein kinase (Boehringer-Mannheim, 1 : 2000); and SMI-32 (Sternberger Monoclonals, 1 : 1000). All antibodies or antisera were diluted in 0.1 M phosphate buffer containing 3% normal serum from the species in which the secondary antibody was raised, 1% bovine serum albumin and 0.25% Triton X-100. After incubation for 12–24 h in the primary antibody at 4°C, sections were washed in phosphate buffer and reincubated in a 1 : 200 solution of biotinylated secondary antibody for 1–2 h at room temperature. They were then washed again and reacted for 1 h in avidin–biotin–peroxidase complex (ABC kit, Vector laboratories), washed and reacted in phosphate buffer containing 0.5 mg ml⁻¹ 3,3' diaminobenzidine 4HCl and 0.01% H₂O₂. Controls consisted of staining selected sections with the same procedure but replacing the primary antibody with preimmune serum. Sections, after staining, were dried, dehydrated, cleared and mounted in DPX. The brains from the archives had all been sectioned in the frontal plane and all sections had been stained with thionin.

Imaging. Stained sections mounted on glass microscope slides were scanned at 20× (0.46 µm per pixel) in a ScanScope T3 scanner (Aperio Technologies, Vista, CA) adapted to accommodate 3"×2" slides. Virtual slides prepared in this way were saved as JPEG-compressed TIFF files and uploaded to a server at www.brainmaps.org (Mikula *et al.* 2007). The 100 000 pixel wide images were down sampled to approximately 10% original size to allow importing into ADOBE PHOTOSHOP for preparation and labelling of figures.

Nuclei recognized as having similar cytoarchitectonic features and relationships to those found in other mammals have been given standardized names (e.g. Jones 2007). Where a nuclear identity has been conjectured, this is indicated by ? before the abbreviation. Divisions of the thalamus that could

Figure 1. (a–f) Nissl-stained frontal sections in (a) anterior to (f) posterior order through the thalamus of a platypus. Scale bar, 1 mm. See [table 1](#) for abbreviations. (*Cont.*)

not be recognized as the equivalents of those in other mammals have been left unlabelled.

3. RESULTS

(a) *Cyto- and myeloarchitecture*

Nissl- and myelin-stained sections from the thalami of both the platypus and the echidna display a clear differentiation of epithalamus, dorsal thalamus and ventral thalamus ([figures 1–5](#)). The platypus thalamus is much larger than that of the echidna and distorted by a relatively enormous, lobe-like, posterolateral expansion of the ventral posterior part of the dorsal thalamus ([figure 1l–t](#)). Unlike in mammals, such as primates, in which posterior enlargement of the lateral posterior–pulvinar complex extends along the lateral surface of the midbrain, in the platypus the enlargement of the thalamus invades and becomes engulfed in the posterior part of the cerebral hemisphere. The echidna thalamus does not present this appearance and remains attached to the lateral aspect of the midbrain separate from the cerebral hemisphere ([figure 2h–l](#)).

The three primary divisions of the thalamus (epithalamus, dorsal thalamus and ventral thalamus) can be readily identified in both species. The greater part of the epithalamus of both species is well defined and consists of clearly distinguishable medial and lateral habenular nuclei; they are relatively anteriorly placed, but have cytological characteristics identical to those of other mammals ([figures 1h–l, 3a,b, 4e–i and 5a–c](#)). The medial habenular nucleus is the larger of the two habenular nuclei; it is made up of closely packed small neurons that tend to form lobulated clumps with relatively acellular intervening spaces. Many of these neurons are immunoreactive for choline acetyl transferase ([Manger *et al.* 2002a](#)). The lateral habenular nucleus is relatively small; it contains both moderately large cells that tend to be concentrated dorsolaterally and smaller cells that tend to be concentrated ventromedially. There is a well-defined stria medullaris ascending the anterior aspect of the thalamus and entering the habenular nuclei, and a large habenulopeduncular tract emerges from their ventral aspects. Paraventricular nuclei are much less

Figure 1. (Continued.)

easy to define than in other mammals. Posteriorly, between the two medial habenular nuclei, there are two small nuclei of small densely packed cells that are merged in the midline, and thus resemble the posterior paraventricular nucleus of other mammals (figure 1*g–k*). Anteriorly, however, the ependyma lining the dorsal recess of the third ventricle is not underlain by the typically compacted, small-celled anterior paraventricular nucleus. Instead, there is a region of neuropil containing only a few scattered cells in that position in the platypus and a zone of compact cells extending up either side of the dorsal recess in the echidna (figures 1*d–f* and 4*a*).

In the ventral thalamus, which is clearly separated from the dorsal thalamus by an external medullary

lamina, there is a distinct reticular nucleus and a zona incerta; these divisions of the ventral thalamus have cytoarchitectures comparable to those in other mammals, but they are anteriorly placed and the reticular nucleus does not encompass the whole lateral aspect of the dorsal thalamus (figures 1*d–i* and 4*a–d*). Throughout its extent, the reticular nucleus is closely apposed to the cells of the external segment of the globus pallidus and, over considerable distances, the cells of the two nuclei are essentially continuous with one another (figure 1*i–t* and see below). In anterior sections, the extended reticular nucleus/external segment of the globus pallidus comes to resemble the transient ‘perireticular nucleus’ of developing mammals (e.g. Hayes *et al.* 2003). The reticular nucleus comes to its end at about the middle of the

Figure 1. (Continued.)

anteroposterior extent of the dorsal thalamus, and from that point on, the cells of the external division of the globus pallidus are directly applied to the surface of the dorsal thalamus in such a manner that the posterior lobe-like expansion of the dorsal thalamus into the cerebral hemisphere in the platypus is surrounded by a thin line of pallidal cells that at first sight resemble the reticular nucleus (figures 1*q–t* and 3*h*). The posterior lobe-like expansion also causes the dorsal thalamus to override the internal segment of the globus pallidus and the substantia nigra; the internal segment of the globus pallidus becomes applied to the dorsal aspect of the internal capsule as it joins the cerebral peduncle so as to appear like the entopeduncular nucleus of many other mammals. The appearance is most marked in the platypus but

evident also in the echidna (figures 1*o–t* and 2, and see below). In the platypus, the cerebral peduncle, substantia nigra and internal segment of the globus pallidus are also drawn up the medial side of the posterior expansion of the thalamus (figure 1*p–t*). In overriding the entopeduncular nucleus and the substantia nigra, the enlarged posterior lobe causes the reticular nucleus, external segment of the globus pallidus, entopeduncular nucleus and pars reticulata of the substantia nigra to merge into a continuous sequence of large, deeply staining cells without well-defined boundaries between the different nuclear components (figure 1*q–t*).

The zona incerta is relatively large, extending from a distinct subparafascicular nucleus and field of Forel medially across the ventral aspect of the dorsal

Figure 1. (Continued.)

thalamus, extending laterally up the side of the latter for a more considerable extent than occurs in other mammals. It too merges with the common reticular nucleus, globus pallidus and pars reticulata mass of cells, especially in the platypus (figure 1*k–m*). Running through the zona incerta towards the posterior aspect of the thalamus in the echidna, but more anteriorly located in the platypus on account of the giant posterior protrusion of the dorsal thalamus, is a commissure not evident in marsupials or eutherian mammals (figure 6). It does not obviously connect any parts of the thalamus; instead, it clearly emerges as a series of bundles from the two segments of the globus pallidus of one side and ends similarly on the other side. It has been named the interpallidal commissure (Jones 2007).

The remaining major division of the ventral thalamus, the ventral lateral geniculate nucleus, is differently located in the platypus and echidna and occupies positions quite dissimilar to its location in other mammals. A nucleus identical to that identified

by the tracing of connections from the eye, and called LGNa by Campbell & Hayhow (1971), is relatively large and situated posteriorly beside the cerebral peduncle in the echidna; it contains large and small cells which can even be partially laminated, resembling the ventral lateral geniculate nucleus of other mammals in all except its pendulous shape and location at the posterior pole of the thalamus. In this posterior position, it extends up the medial side of the posterior pole of the dorsal thalamus to meet the lateral surface of the superior colliculus (figures 1*p,q* and 7). In the platypus, the whole ventral lateral geniculate nucleus, which is relatively large and partially laminated with alternating large- and small-celled regions as in the echidna, is displaced dorsomedially and lies adjacent to the anterior pole of the superior colliculus (figures 1*p,q* and 7). Like the dorsal lateral geniculate nucleus (see below), it can be more confidently regarded as the ventral lateral geniculate nucleus by tracing the optic tract to it (Campbell & Hayhow 1971). The optic tract

Figure 1. (Continued.)

of the echidna can be traced to the ventral lateral geniculate nucleus and then continues as a medially directed arm that follows the dorsally extending ventral lateral geniculate nucleus to the brachium of the superior colliculus, and as a lateral arm, located on the dorsolateral aspect of the dorsal thalamus and ending in the dorsal lateral geniculate nucleus (figures 1*m–t*, 2*h–m*, 3*f*, 4*g–l*, 5*h–j*, 7 and 8). In the platypus, the study of Campbell & Hayhow (1972) on the retinal projections in the platypus is key to identifying the optic tract and the ventral and dorsal lateral geniculate nuclei, for the enormous posterior outgrowth of the dorsal thalamus has occurred in such a manner as to displace the whole optic tract medially in relation to the dorsal thalamus. The large ventral lateral geniculate nucleus thus lies in an uncharacteristic position dorsolaterally adjacent to the superior colliculus (figures 1*m*, 7 and 8). The fibres of the optic tract ascend to the brachium of the superior colliculus along a trajectory similar to that of the echidna, but this

bundle of fibres also contains fibres destined for a tiny dorsal lateral geniculate nucleus located lateral to the dorsally displaced ventral lateral geniculate nucleus (figures 1*m*, 7*a*, 8 and see below).

In both the platypus and the echidna, the dorsal thalamus presents an initial impression of a lack of nuclear differentiation even in comparison with the least differentiated thalami of marsupials or eutherian mammals. On close inspection, however, the cellular architecture of the dorsal thalamus is by no means homogeneous in either species.

In the dorsal thalamus of both species, an internal medullary lamina is not present so anterior, medial and ventral/lateral divisions of the dorsal thalamic nuclei cannot be delineated by their relationships to an internal medullary lamina, and no groups of cells that could be regarded as the equivalents of the intralaminar nuclei can be identified with certainty. The fibre tracts that normally contribute to the lamina in other mammals become markedly dispersed on entering the

Figure 1. (Continued.)

thalamus. The mamillothalamic tract, although large in the monotremes, begins to splay out in small bundles immediately on entry into the thalamus (figure 2*b–e*). These small bundles radiate through the anterior region of the dorsal thalamus (figures 2*c,d* and 5*b*) but, in failing to consolidate as an internal medullary lamina, do not isolate a population of cells that can be immediately identified as the anterior group of dorsal thalamic nuclei. Scattered among the bundles of the mamillothalamic tract are cells whose architecture resembles that of the anteroventral nucleus of other mammals and we identify this region as the anterior nuclear complex, but there is no differentiation into anteroventral, anteromedial or anterodorsal nuclei typical of therian mammals. At the anterior pole of the thalamus and closely applied to the undersurface of the stria medullaris (taenia thalami) is a population of cells whose architecture resembles that of the parataenial nucleus of rodents and marsupials (figure 1*c–e*). Fibre bundles entering the dorsal thalamus from the internal capsule dorsally and posteroventrally also tend to splay out immediately on

entry (figures 2, 6 and 7*f*) and these, too, fail to contribute to an internal medullary lamina.

Ventromedially in the anterior part of the dorsal thalamus, located on either side of the third ventricle, are paired masses of cells that are slightly more densely packed than elsewhere. These merge in the midline, to form a bilobed structure resembling a nucleus reuniens of other mammals (figures 1*d–g*, 3*a–d*, 4*a,b* and 5*a*). Hence, we call these nuclei individually the medioventral nucleus and the fused nuclei the nucleus reuniens, following the practice introduced by Berman & Jones (1982).

As the dorsal thalamus expands posteriorly, there is a large region of dorsally located, relatively dispersed cells that are not well delineated from adjacent cell populations but, as described below, are all calbindin immunoreactive; this is a feature of the lateral dorsal nucleus of other mammals and we accordingly call the region by this name. Medially, there is a rounded region of slightly more densely stained cells that might be considered a mediodorsal nucleus, but it is not surrounded by intralaminar or midline nuclei and thus its borders are ill-defined. We have labelled it M for

Figure 2. (a–m) Myelin-stained frontal sections from the same brain as in figure 1, arranged in (a) anterior to (m) posterior order. Scale bar, 1 mm. See table 1 for abbreviations. (Cont.)

medial nuclear complex in the figures (figures 1–6). Posteriorly, this putative mediodorsal nucleus is penetrated by the habenulopeduncular tract, around which the cells become a little more densely stained and, on the basis of its relationship to the tract and the density of cell staining, this region can be provisionally identified as a parafascicular nucleus (figures 1*k,l*, 3*a,b*, 4*f,g*, 5*d,e* and 6*a*).

At the region at which the interpalldal commissure crosses the midline, the medioventral nucleus gives way to a quite distinct region of slightly larger cells, which is provisionally identified as a ventral medial nucleus (figures 1*h–j*, 3*d*, 4*c–f*, 5*b–d* and 6). Extending laterally from this and along the commissure is a thin zone of larger, more deeply staining cells, which expands posterolaterally (figures 1*k–n* and 4*d–g*). In the platypus, with the downward growth of the ventral posterior nucleus, this line of cells is drawn up medially along the anterior pretectal nucleus and it comes to occupy a small triangular region at the boundary between dorsal thalamus and pretectum that might represent a limitans–suprageniculate nucleus. In the echidna, it is more rounded in shape.

Cytoarchitectonic differentiation is far greater in the posterior regions of the dorsal thalamus of both

monotremes than in the anterior regions. The ventral posterior nucleus is the most distinct (figures 1*l–t*, 3*f–h*, 4*d–i* and 5*h–k*). In both species, it lies along the interpalldal commissure anteriorly and extends posteriorly as a large, multilobular, ovoid structure in the echidna and as an enormous, laterally extended, pear-shaped structure in the platypus. The ventral posterior nucleus of the echidna extends back towards the midbrain in a manner in which anatomical relationships between the thalamus and midbrain can be recognized as distinctly similar to those found in other mammals. In the platypus, however, the extreme enlargement of the ventral posterior nucleus causes the dorsal thalamus to invade the cerebral hemispheres and to continue within them to a level well posterior to that at which the hemispheres separate from the diencephalon (figures 1*t* and 3*g,h*).

The architecture of the ventral posterior nucleus is similar in both monotremes. There are three incompletely separated divisions (figures 1*l–t* and 4*d–i*). Medially is a region of relatively small, densely packed cells, which resembles the basal ventral medial nucleus of other mammals. Anteriorly, it lies along the interpalldal commissure, but posteriorly it expands up the lateral side and over the posterior pole of the ventral posterior

Figure 2. (Continued.)

nucleus. In the platypus, the region is streaked by three or more myelinated fibre bundles running across the nucleus with a dorsolateral to ventromedial orientation (figure 2*i–m*). These streaked bundles are not associated with any associated differences in cell packing density or cell type within the nucleus. In the echidna, the small-celled part of the ventral posterior nucleus is very highly lobulated without the fibre streaking (figures 4*g–i* and 5*h–j*). Ventromedially, the medial lemniscus is large and multifasciculated, with fascicles radiating into the ventral posterior nucleus; a large medial bundle within the lemniscus of the platypus suggests an ipsilateral trigeminothalamic tract (figures 1*n–q*, 2*j–m*, 3*f,g*, 4*g–k* and 5*f–h*).

The other two divisions of the ventral posterior nucleus are more evident in the platypus than in the echidna. There is a central region of somewhat less densely packed cells and a dorsal region of larger, somewhat more dispersed cells. In neither animal can

an arcuate lamella be discerned and no divisions resembling ventral posterior medial and ventral posterior lateral nuclei can be identified.

Anterior to the ventral posterior nucleus is a region of relatively large, less densely packed cells that possibly represents a ventral lateral nucleus (figures 1*f–p*, 3*e–h*, 4*c–e* and 5*h–k*). It has been labelled accordingly. Dorsal to it, the lateral dorsal nucleus gradually tapers to an end and is replaced by a larger region of slightly more densely packed cells; by exclusion, we will call this the lateral posterior nucleus. It is quite large in both species of monotreme but it progressively narrows and disappears as the ventral posterior nucleus expands to fill the whole posterior aspect of the thalamus in the platypus, but it extends over the posterior pole of the dorsal thalamus in the echidna.

In neither species can a medial geniculate complex be identified with any certainty. Fibres forming a

Figure 3. (a–h) Nissl-stained parasagittal sections in (a) medial to (h) lateral order through the thalamus of a platypus. Scale bar, 1 mm. See [table 1](#) for abbreviations. (*Cont.*)

brachium of the inferior colliculus can be seen entering the diencephalon from the midbrain, but they tend to disappear into the posterior pole of the ventral posterior nucleus in the echidna and into the ventromedial aspect of the same nucleus in the platypus. It is possible that some of the cell lobules in the ventral aspect of the echidna ventral posterior nucleus ([figures 4h–k](#) and [5i–l](#)) could represent a medial geniculate complex (identified by ?MG in the figures; [Campbell & Hayhow 1971](#)). No similar groups can be identified in the platypus.

A dorsal lateral geniculate nucleus ([figure 1m](#)) can be identified on the dorsal aspect of the platypus thalamus as described above. It is very small and only recognizable by following the optic tract to it ([figures 1m](#) and [7a](#); [Campbell & Hayhow 1972](#)). [Hines \(1929\)](#) mistook it for the bed nucleus of the stria terminalis which lies immediately lateral to it but separated from it by the vena terminalis ([figure 8](#)). In the echidna, the dorsal lateral geniculate nucleus is dorsolaterally

located as in many other mammals; it is much larger than in the platypus, and its cells tend to form lobular masses that posteriorly merge with those of the more distinct putative lateral posterior nucleus ([figures 4i–j](#) and [5j–l](#)); it descends over the posterior pole of the echidna thalamus towards the ventral lateral geniculate nucleus, so that the relative positions of the two geniculate nuclei there become the same as in marsupials and non-anthropoid mammals. The massive growth of the ventral posterior nucleus in the platypus prevents a comparable union and the ventral lateral geniculate nucleus, which is relatively large and partially laminated with alternating large- and small-celled regions, is displaced to the lateral edge of the superior colliculus ([figures 1p,q](#) and [7a–c](#)).

(b) Chemical anatomy

Acetyl cholinesterase staining does not provide evidence of an incipient internal medullary lamina in either species. Staining is absent throughout all parts of the

Figure 3. (Continued.)

thalamus with the exception of the habenular nuclei, habenulopeduncular tract and in a thin lamina within the ventral lateral geniculate nuclei (figure 9*a–d*).

Parvalbumin and calbindin are expressed in thalamic neurons of both the platypus and the echidna; as in other mammals, they are expressed in different populations of neurons (figure 10). Regions containing large populations of parvalbumin immunoreactive cells are associated with enhanced cytochrome oxidase staining (not shown). Regions containing large populations of calbindin immunoreactive cells are associated with weak cytochrome oxidase staining as in other mammals (Rausell & Jones 1991*a,b*; Rausell *et al.* 1992; Jones 1998). Parvalbumin immunoreactivity is characteristic of all cells in the reticular nucleus of the two monotremes and is found in many cells of the zona incerta in both, as well as in the cells of the substantia nigra pars reticulata and the two divisions of the globus pallidus (figures 10–12). In the dorsal thalamus, there are no parvalbumin immunoreactive cells anteriorly, except for a small number of well-stained cells in the putative anterior nuclear complex (figure 11*a*). These

look remarkably similar to a group identified as invaders from the reticular nucleus in other mammals (Jones 2007). The number of parvalbumin immunoreactive cells increases posteriorly, and a large population fills the ventral lateral and ventral posterior nuclei and the overlying putative lateral posterior nucleus (figure 11*c*). Throughout the length of the dorsal thalamus, however, there are no parvalbumin immunoreactive cells in medial regions, including in the putative parafascicular nucleus, and few or none in the lateral dorsal nucleus (figures 10, 11*b,c* and 12*d*).

In the ventral posterior nucleus, there are many densely parvalbumin immunoreactive smaller cells embedded in a dense parvalbumin immunoreactive neuropil. Extending across the ventral posterior nucleus from dorsolateral to ventromedial, there are four bands of enhanced parvalbumin neuropil immunoreactivity (figure 11*c*). Two are located anteriorly in the nucleus and two posteriorly. These are the same bands that display enhanced staining for myelin (see above), and also display enhanced staining for cytochrome oxidase, calbindin and GABA. The fibres

Figure 4. (a–f) Frontal sections in (a) anterior to (f) posterior order through the thalamus of an echidna. All sections are Nissl stained except (b), which is myelin stained. Scale bar, 1 mm. See [table 1](#) for abbreviations. (Cont.)

of the medial lemniscus are immunostained for parvalbumin, and this reveals its very large size and extent in relation to the ventromedial aspect of the dorsal thalamus.

In the epithalamus, the medial habenular nucleus contains only a few parvalbumin immunoreactive cells. In the ventral thalamus, the reticular nucleus and zona incerta possess many parvalbumin cells that are continuous with similarly immunoreactive cells in the two segments of the globus pallidus and pars reticulata of the substantia nigra ([figure 11c](#)); the ventral lateral geniculate nucleus, however, has few or none.

Cell populations exhibiting calbindin immunoreactivity in the monotreme thalamus are largely complementary to those displaying parvalbumin immunoreactivity ([figures 10–12](#)). The region identified as the

anterior nuclear complex contains many densely packed calbindin immunoreactive cells, and the region identified as the lateral dorsal nucleus is particularly densely packed with them ([figure 12a–c](#)). This high density of calbindin cells serves to outline a large lateral dorsal nucleus that extends across most of the mediolateral extent of the dorsal thalamus and well back towards the posterior pole before being replaced by the putative lateral posterior nucleus, in which there are smaller numbers of more weakly calbindin immunoreactive cells and an equal population of parvalbumin immunoreactive cells as well ([figure 10h](#)). The putative medial, medioventral and ventral medial nuclei contain many calbindin immunoreactive cells ([figures 10 and 12a–c](#)), but the number in the ventral posterior nucleus is small and neuropil staining is weak, apart from that in the oblique bands

Figure 4. (Continued.)

described above. Outside the dorsal thalamus, calbindin immunoreactive cells are found in the zona incerta, posterior paraventricular nucleus, as a band in the dorsolateral aspect of the medial habenular nucleus, and as a small group in the dorsomedial corner of the ventral lateral geniculate nucleus (figure 10*c–e,i*).

Immunoreactivity for GABA is widespread in cells of the monotreme thalamus. All cells of the reticular nucleus and some in the zona incerta and ventral lateral geniculate nucleus are GABA immunoreactive (figure 13). Those of the external segment of the globus pallidus merge with and replace those of the reticular nucleus on the posterodorsal aspect of the thalamus, and in the platypus encircle the huge posterior extension of the dorsal thalamus into the cerebral hemisphere. These GABAergic cells also form the continuum extending from reticular nucleus through the external segment of the globus pallidus

and entopeduncular nucleus into the pars reticulata of the substantia nigra described above. In the dorsal thalamus, the pattern of cellular immunoreactivity for GABA follows closely that of parvalbumin; there are a few well-stained GABA cells in the putative anterior nuclei, and their numbers increase on going posteriorly in the lateral parts of the thalamus. There are no GABA immunoreactive cells in the medial regions of the dorsal thalamus.

Immunoreactivity for tyrosine hydroxylase, serotonin and choline acetyl transferase reveals permeation of the dorsal thalamus by fibres immunoreactive for these epitopes, and there are higher densities of immunoreactive fibres in the reticular nucleus and zona incerta and in the (posterior) paraventricular nucleus (not shown). No accumulation of immunoreactive fibres that could be construed as a nascent internal medullary lamina could be detected, but there was a strong

Figure 5. (a–f) Nissl-stained parasagittal sections in (a) medial to (f) lateral order through the thalamus of an echidna. Scale bar, 1 mm. See [table 1](#) for abbreviations. (Cont.)

tendency for tyrosine hydroxylase-immunoreactive fibres to form a plexus in the medial regions of the dorsal thalamus ([figure 13d](#)). The only choline acetyl transferase-immunoreactive cells are located in the medial habenular nucleus ([Manger et al. 2002a](#)). Neurons immunoreactive for choline acetyl transferase, serotonin or tyrosine hydroxylase, from which the afferents to the monotreme thalamus presumably arise, are located in brainstem nuclei comparable to those of other mammals ([Manger et al. 2002a–c](#)).

4. DISCUSSION

The cyto- and chemoarchitecture of the monotreme thalamus, when examined in depth, reveals far more similarities to the thalami of marsupial and placental

mammals than a cursory glance at a Nissl-stained preparation suggests. The homologues of ventral, dorsal and epithalamus are clearly evident and organized in a manner fundamentally similar to that of metatherian and eutherian mammals, and these divisions have similar immunocytochemical characteristics. The foreshortening of the reticular nucleus, its continuity with the globus pallidus and its replacement by the GABA cells of the external segment of the globus pallidus posteriorly, and the obvious continuity between the GABA cells of the ventral thalamus, globus pallidus and substantia nigra are unique features of the monotreme diencephalon. In the epithalamus, the difficulty in identifying an anterior paraventricular nucleus, especially in the platypus, is puzzling but otherwise the divisions of the epithalamus are very

Figure 5. (Continued.)

similar to those of other mammals. Lobulation of the medial habenular nucleus is found in many other mammals and large- and small-celled populations can also usually be identified in the lateral habenular nucleus of other mammals, becoming so well defined in the primates that they reach the status of separate subnuclei (Jones 2007).

The dorsal thalamus of the monotremes shows the greatest variation from other mammals until examined in depth and with the benefit of complementary immunocytochemical staining for the calcium-binding proteins. The lack of an internal medullary lamina, which seems to be determined by an unusual splaying out of fibres entering the thalamus from major fibre tracts such as the mamillothalamic, is a cause of uncertainty in making

nuclear definitions in the anterior and medial regions of the dorsal thalamus. However, immunocytochemical staining patterns and the enclosing of the anterior polar region by the splayed out fascicles of the mamillothalamic tract suggest the presence of an anterior group or complex of nuclei. The lack of an enfolding arm of the internal medullary lamina may have influenced Ashwell & Paxinos (2005) in referring to the region we call the anterior nuclei as the parataenial nucleus in the echidna. The lack of identifiable anterior nuclei, coupled with the relatively enormous size of the frontal cortex in the echidna, influenced Regidor & Divac (1987) in identifying the anterior region of the echidna thalamus as all belonging to the mediodorsal nucleus. This whole anterior region undergoes retrograde degeneration after destruction of the frontal cortex in the echidna

(Welker & Lende 1980), but the lesions could have encompassed the target cortex of anterior nuclei projections as well as those of a mediodorsal nucleus equivalent, the one equivalent to cingulate cortex and the other to prefrontal cortex.

The calbindin immunostaining pattern makes identification of a lateral dorsal nucleus, even in the absence of an unmistakable cytoarchitecture, almost incontrovertible owing to the consistency with which this nucleus expresses calbindin in all mammals that have been studied (Jones 2007). Medioventral and ventral medial nuclei are distinguishable on cytoarchitectonic grounds and the former on the basis of calbindin immunoreactivity as well.

The lack of cell groups that might be regarded as the equivalents of the intralaminar system, other than a somewhat indistinct parafascicular nucleus, is puzzling. Given the presence of a striatum of significant size, one would anticipate the existence of a substantial thalamostriatal projection, which in other mammals arises predominantly from cells located in intralaminar nuclei and their midline extensions. Conceivably, the tyrosine hydroxylase immunoreactive plexus illustrated in figure 13*d* identifies a region of striatally projecting

cells, since the internal medullary lamina of other mammals usually contains a high concentration of such fibres.

In the posterior and lateral parts of the dorsal thalamus, the ventral posterior nucleus stands out very clearly in both monotremes and over it can be seen a nucleus different in immunocytochemical characteristics from the lateral dorsal nucleus and, therefore, likely to be a lateral posterior nucleus. The enormous size of the trigeminal nuclei and the medial lemniscus in both monotremes, especially the platypus, suggests that a substantial part of the ventral posterior nucleus should be devoted to the representation of the bill of the platypus and the beak of the echidna. However, no division of the ventral posterior nucleus into subnuclei comparable to the ventral posterior medial (trigeminal) and ventral posterior lateral (rest of the body) representations could be discerned. The densely packed small-celled medial part of the ventral posterior nucleus suggests a basal ventral medial (taste and visceral) nucleus (Jones 1985, 2007).

In the echidna, regions that include the ventral posterior nucleus described here project to a